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Original Research

Unveiling the Impact of Thiophanate-Methyl on *Arthrospira platensis*: Growth, Photosynthetic Pigments, Biomolecules, and Detoxification Enzyme Activities

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Abstract

Background: The intensive and injudicious use of pesticides in agriculture has emerged as a major concern due to its detrimental impact on aquatic ecosystems. However, the specific impact of broad-spectrum fungicides, such as Thiophanate-methyl (TM), on cyanobacteria remains a subject of ongoing research and debate. **Methods:** In order to fill this knowledge gap, the present study aimed to comprehensively investigate the toxicological effects of TM (10–30 µg/L) on the growth, photosynthetic pigments, oxidative stress, and biochemical composition of the non-nitrogen-fixing cyanobacterium *Arthrospira platensis*. **Results:** Our findings unequivocally demonstrated that TM exposure significantly inhibited the growth of *A. platensis*. Moreover, the decrease in chlorophyll content indicated a pronounced negative impact on the photosynthetic system of *A. platensis* caused by TM exposure. Notably, TM induced oxidative stress in *A. platensis*, as substantiated by a significant increase in lipid peroxidation (MDA) within the culture. Furthermore, the intracellular generation of hydrogen peroxide (H₂O₂) exhibited a positive correlation with higher TM dosages, while the levels of vital antioxidant enzymes, such as catalase (CAT) and ascorbate peroxidase (APX), exhibited a discernible decrease. This suggests that TM compromises the antioxidant defense mechanisms of *A. platensis*. Additionally, TM was found to enhance the activity of a plethora of enzymes involved in the detoxification of pesticides, including peroxidase (POD) and glutathione-S-transferase (GST), thereby indicating a robust detoxification response by *A. platensis*. Interestingly, exposure to TM resulted in a general suppression of biocomponent production, such as total proteins and total carbohydrates, which exhibited a diminishing trend with increasing TM concentration. Conversely, the lipid content witnessed a significant increase, possibly as an adaptive response to TM-induced stress. **Conclusions:** These findings contribute to a deeper understanding of the ecological implications of pesticide usage and emphasize the urgent need for the adoption of sustainable and environmentally-friendly agricultural practices to safeguard aquatic ecosystems.

Keywords: cyanobacteria; *Arthrospira platensis*; thiophanate-methyl; oxidative stress; ecotoxicology

1. Introduction

In agriculture worldwide, fungicides are extensively employed to control the proliferation of unwanted noxious plants. However, the use of these pesticides in large quantities raises significant concerns due to their potential toxicity. When used excessively, fungicides can have detrimental effects on agricultural soil, plants, aquatic life, and human organisms. Among the biocides utilized to inhibit the growth of fungi, bacteria, and parasites in crops, animals, and humans, the benzimidazole fungicides group is widely prevalent [1]. One specific member of this group is Thiophanate methyl (TM), which serves as a systemic fungicide extensively utilized in agriculture to protect crops

against fungal diseases [2]. TM is classified as a grade IV or low-toxicity chemical, indicating a relatively lower risk to human health and the environment. However, even though it falls into this category, TM can still be detected in soil samples as well as in vegetables and fruits, classifying it as a “pesticide residue” of growing concern. Consequently, there is an increasing need to investigate the potential ecological and health impacts associated with the presence of TM residues [3]. Benzimidazole fungicides harmfully affect soil microbial activity, such as rhizosphere microbes diversity, abundance and composition, all of which impact microbial mineralization of organic molecules, nutrient cycling and bioavailability, pollutants detoxification, and xenobiotic bioremediation, leading to soil degradation



and yield loss [4,5]. Furthermore, TM can enter aquatic ecosystems through water runoff, posing a serious threat to freshwater microalgae. These microorganisms play a vital role in the ecosystem as they contribute to oxygen production and act as a crucial food source for other organisms. Therefore, the pollution of aquatic environments with TM can disrupt the balance of the ecosystem and have far-reaching consequences [3]. Studies have revealed the adverse effects of TM exposure on various organisms. For instance, TM has been found to induce developmental and notochord toxicity in zebrafish (*Danio rerio*) embryos [6]. This highlights the potential risks associated with TM exposure, even at early stages of development. However, the specific toxicological effects of TM on *Spirulina platensis*, a significant prokaryotic microalgae species, remain largely unexplored [6].

Photoautotrophic organisms such as green algae and cyanobacteria are the base of the food web in aquatic ecosystem. They provide a large amount of oxygen and food for other organisms and affect the stabilization of the hole ecological system [7]. Moreover, they play an essential role in minerals cycling. *Spirulina platensis*, a member of the cyanobacteria phylum, stands out as a significant prokaryotic microalgae species among several others. Its extensive usage by humans over an extended period of time can be attributed to its remarkable nutritional value. With protein constituting around 60% of its dry weight, *Spirulina platensis* is rich in essential components like amino acids, vitamins, minerals, and unsaturated fatty acids [8]. Recently, it was found that *Arthrospira platensis* (formerly known as *Spirulina platensis*) was very sensitive to the environmental pollutants [9]. For example, research conducted by Jiang *et al.* [10] demonstrated the toxicological effects of antibiotics norfloxacin (NFX) and sulfadiazine (SDZ) on *Arthrospira platensis*. Furthermore, a study by Abbasi *et al.* [11] revealed that polyethylene microplastics induced adverse effects on the growth and photosynthetic activity of *Spirulina*. These effects included oxidative stress, fragmentation, and swelling of trichomes. Such findings emphasize the importance of studying the influence of pesticides and other emerging pollutants on microalgal growth and population dynamics. By gaining insights into the ecotoxicological risks associated with these substances, it becomes possible to assess and mitigate their potential harm to ecosystems [11]. Furthermore, the toxicological effect of TM on the *Spirulina platensis* remains unclear.

In the present study, our main objective was to investigate the intricate relationship between pesticide residues, specifically the fungicide TM, and their impact on aquatic ecosystems. We focused on examining the influence of different dosages of TM on various physiological parameters using *Arthrospira platensis* as the model organism. This choice was based on the significance of *Arthrospira platensis* in understanding the response of primary producers in aquatic environments. TM is a commonly de-

tected pesticide residue in aquatic ecosystems, making it a critical area of research due to its potential ecological hazards. Our study aimed to provide comprehensive insights into the impact of TM and other pesticide residues on the delicate balance of aquatic ecosystems. By specifically exploring the effects of TM on *Arthrospira platensis*, we aimed to shed light on the potential risks and threats posed by these residues to the stability and health of aquatic environments. Understanding the ecological implications of pesticide residues, including TM, is of paramount importance due to their persistence in water bodies and far-reaching consequences for the organisms inhabiting these ecosystems. Our research contributes to the growing body of knowledge concerning pesticide residue pollution in aquatic ecosystems. It also highlights the urgent need to adopt sustainable and environmentally-friendly agricultural practices to mitigate the risks associated with pesticide residues. Through a comprehensive analysis of various physiological parameters of *Arthrospira platensis*, such as growth, photosynthetic pigments, oxidative stress, and biochemical composition, we aimed to unravel the complex interactions between pesticide residues and microalgae. This knowledge is essential for developing effective strategies to mitigate the adverse effects of pesticide residues on aquatic ecosystems. Ultimately, our study seeks to ensure the long-term sustainability and health of our water resources.

This work represents a novel contribution as it explores a research area that has not been previously investigated. The specific investigation of the influence of different dosages of the fungicide TM on various physiological parameters of *Arthrospira platensis* in the context of aquatic ecosystems is a unique aspect of this study. By examining its growth, photosynthetic pigments, oxidative stress, and biochemical composition, the study offers a detailed understanding of how pesticide residues, including TM, affect this microalgae. By providing detailed information on the specific effects of TM on *Arthrospira platensis*, the study enables a more accurate assessment of the associated risks. It also emphasizes the need for effective environmental management measures to mitigate these risks. Lastly, the findings of this study have important implications for sustainable management practices. By highlighting the significance of environmentally friendly agricultural practices, the article underscores the importance of reducing risks associated with pesticide residues to preserve the long-term health and sustainability of aquatic ecosystems.

2. Materials and Methods

2.1 Chemicals

Commercial formulation of Thiophanate-methyl (70% waters dispersible granule) as active ingredient, was used as test substance in the study. It is purchased from local market of Medea, Algeria. The selection of fungicide was based on a survey conducted in Medea province in 2022, which indicated that Thiophanate-methyl was the

most widely used active ingredient pesticides in the region. All other reagents and solvents used were of analytical grade.

2.2 Microalgae Cultivation and Growth Inhibition Tests

In this study, the selection of cyanobacteria *Arthrospira platensis* as the test species holds significant importance. This particular strain of cyanobacteria was collected from a sebkha located in the Sahara region of Tamanrasset, Algeria, representing a unique ecological niche. By using this specific strain, the study aims to capture the responses and adaptations of *Arthrospira platensis* to the fungicide TM under controlled laboratory conditions. To facilitate the cultivation of *Spirulina*, 500 mL Erlenmeyer flasks were utilized, each containing sterilized Zarrouk culture medium. The pH of the culture medium was carefully adjusted to 9.1 ± 0.03 using HCl and NaOH [12]. This adjustment ensures optimal growth conditions for *Arthrospira platensis*. The initial inoculum quantity was determined to achieve a biomass concentration of approximately 0.513 ± 0.028 g/L, allowing for a standardized starting point for the experiments. In order to assess the impact of TM on *Arthrospira platensis*, cultures in the logarithmic growth phase were subjected to different concentrations of TM for a duration of four days. Specifically, concentrations of TM ranging from 10 to 30 $\mu\text{g/L}$ were employed, providing a range of exposure levels that reflect potential real-world scenarios. A control group, consisting of cultures grown in Zarrouk medium without the addition of TM, was included to establish a baseline for comparison.

Throughout the experimental period, the cultures were maintained at a constant temperature of 34 ± 3 °C. To simulate natural light conditions, an alternating 12-hour light-dark cycle was implemented. During the 12-hour light period, a white fluorescent light with an intensity of $3.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ was used to provide illumination for photosynthesis. The subsequent 12-hour dark period allowed for metabolic processes and recovery. To prevent aggregation and ensure homogenous light exposure, the cultures were manually shaken three times a day. Additionally, the placement positions of the cultures were regularly changed to ensure equal light distribution among the flasks. These measures were taken to minimize any potential bias that could arise from uneven lighting or cell settling. To enhance the statistical reliability and validity of the results, all treatments were performed in quadruplicate. This quadruplicate setup enables the assessment of inter-sample variability and strengthens the statistical significance of the obtained data. By maintaining constant experimental conditions and replicating the treatments, the study aims to provide robust and reproducible results.

2.3 Morphological Analysis

2.3.1 Determination of the Biomass of *A. platensis*

In order to accurately assess the biomass of *Arthrospira platensis*, the dry cell weight (DCW) was utilized as a quantitative measure. The DCW serves as a reliable indicator of the cellular mass of the cyanobacterial culture and is commonly used in such studies. To determine the DCW, optical density measurements at a specific wavelength were employed.

In this study, the optical density of the cultures was measured at 618 nm, a wavelength commonly used for assessing the growth and biomass of *Arthrospira platensis*. The optical density is proportional to the concentration of cells in the culture, allowing for the estimation of biomass. To convert the measured absorbance values to dry weight, a conversion equation was applied. This equation takes into account the relationship between the optical density and the actual dry weight of the cells. The specific conversion equation used in this study was determined based on previous calibration experiments and validation studies. By applying following equation, the optical density measurements obtained at 618 nm were transformed into corresponding dry weights.

$$y = 1.1482 \times + 0.085 (R^2 = 0.983)$$

2.3.2 Determination of the Growth Inhibition Ratio (IR)

The impact of different dosages of the fungicide TM (ranging from 0 to 30 $\mu\text{g/L}$) on the growth and physiological parameters of *Arthrospira platensis* was assessed using the IR. The IR provides a quantitative measure of the extent to which TM inhibits or affects the biological processes of the cyanobacterium [13].

To calculate the IR, the following formula was utilized:

$$\text{IR (\%)} = (1 - T/C) \times 100\%$$

Where:

IR = Inhibition Rate

C = Control Value (physiological parameter without TM exposure)

T = Test Value (physiological parameter with TM exposure)

In this study, various physiological parameters of *A. platensis* were assessed, such as growth rate, photosynthetic pigments, oxidative stress markers, and biochemical composition. The IR was calculated for each parameter at different TM dosages. The IR values indicate the degree of inhibition or alteration of each physiological parameter caused by TM exposure. A higher IR signifies a greater impact of TM on the specific parameter, indicating increased sensitivity or susceptibility of *A. platensis* to the fungicide.

2.4 Biochemical Analysis

2.4.1 Determination of the Photosynthetic Pigments Contents

To determine the content of chlorophyll a, chlorophyll b, and carotenoids in *Arthrospira platensis*, a solvent extraction method using 3 mL of 96% ethanol was employed. The extraction process allowed for the release and solubilization of these photosynthetic pigments present in the cyanobacterial cells. Following the extraction, the concentrations of chlorophyll a, chlorophyll b, and carotenoids were quantified using spectrophotometric measurements at specific wavelengths. The absorbance of the extracted pigment solution was measured at 665 nm for chlorophyll a, 649 nm for chlorophyll b, and 470 nm for carotenoids. To calculate the concentration of photosynthetic pigments, a mathematical equation previously described by a relevant source [14] was applied. This equation takes into account the absorbance values obtained from the spectrophotometer and allows for the determination of the pigment concentrations in the samples. By utilizing this equation, the researchers were able to accurately quantify the content of chlorophyll a, chlorophyll b, and carotenoids in *Arthrospira platensis* cultures exposed to different TM dosages. This provided valuable information on the impact of TM on the photosynthetic apparatus and pigment composition of the cyanobacterium.

$$\text{Chl}_a (\text{mg/L}) = \frac{(13.95 A_{665} - 6.88 A_{649}) \times V_2}{V_1}$$

$$\text{Chl}_b (\text{mg/L}) = \frac{(24.96 A_{649} - 7.32 A_{665}) \times V_2}{V_1}$$

$$C_{\text{carotenoid}} (\text{mg/L}) = \frac{(1000 A_{470} - 2.05 C_a - 114.8 C_b) \times V_2}{245 \times V_1}$$

V_1 and V_2 : volumes of sampled *A. platensis* suspension and latter supernatant, respectively.

A_{665} , A_{649} and A_{470} are the absorbance of the supernatant at 665, 649 and 470 nm, respectively.

Chl_a , Chl_b , and $C_{\text{carotenoid}}$ indicate the concentration of chlorophyll a, chlorophyll b, and carotenoid, respectively.

2.4.2 Determination of Oxidative Stress Biomarkers

Malondialdehyde (MDA) is a well-known biomarker used to assess lipid peroxidation, which is indicative of oxidative stress in cells. In this study, the content of MDA in *A. platensis* was determined as a measure of lipid peroxidation caused by TM exposure. To quantify the MDA content, a method described by a relevant source [15] was followed. The procedure involves the reaction of MDA with thiobarbituric acid (TBA) to form a colored complex, which can be measured spectrophotometrically. The MDA content was then expressed as micromoles per gram of fresh weight ($\mu\text{mol/g FW}$), providing a quantitative measurement of lipid peroxidation in the cyanobacterial cells.

Another parameter assessed to evaluate the oxidative stress induced by TM exposure was hydrogen peroxide (H_2O_2) content. H_2O_2 is a reactive oxygen species that can cause cellular damage and is commonly associated with ox-

idative stress. The determination of H_2O_2 content was performed following a protocol outlined by a relevant source [16]. This method involves the reaction of H_2O_2 with a specific reagent to produce a colored compound, the intensity of which can be measured spectrophotometrically. The resulting H_2O_2 content provides insights into the oxidative stress status of *A. platensis* cells exposed to TM.

2.4.3 Determination of the Antioxidants System

To obtain the cell lysate for antioxidant and detoxification enzyme assays in *A. platensis*, a series of steps were followed. Initially, aliquots of the microalgae suspension, approximately 10 mL in volume, were collected. These aliquots were then subjected to centrifugation at 4 °C for 15 minutes at 15,000 rpm. The centrifugation step aimed to separate the microalgal pellets from the suspension. Following centrifugation, the microalgal pellets were washed multiple times with distilled water to remove any impurities or contaminants. The washing process involved repeated cycles of centrifugation and resuspension. After the final wash, the microalgal pellets were resuspended in 1 mL of 0.1 M Tris-HCl buffer with a pH of 7.4. To disrupt the microalgal cells and release the cellular contents, the resuspended microalgal pellets were subjected to sonication. The sonication process was carried out at 4 °C for 5 minutes. Subsequently, the sonicated samples were centrifuged at 4 °C for 10 minutes at 15,000 rpm to separate the cell debris from the cell lysate. The supernatant obtained after centrifugation, which contained the cell lysate, was collected for further analysis of antioxidant and detoxification enzyme activities [14]. The specific enzymes measured in this study included catalase (CAT), ascorbate peroxidase (APX), glutathione-S-transferase (GST), and peroxidase (POD). The assay methods for these enzymes were performed according to previously described protocols [17–20], respectively.

The enzyme activities were quantified and expressed as units per milligram of protein (U/mg protein), providing a measure of the enzyme's catalytic efficiency. These enzyme activities serve as indicators of the antioxidant and detoxification capacities of *A. platensis* in response to TM exposure.

2.4.4 Determination of Primary Biocomponents

To assess the concentration of free carbohydrates in the biomass of *A. platensis*, the Anthrone method was employed [21]. Glucose was used as the standard for calibration. The biomass, obtained from 5 mL of the culture, was subjected to centrifugation at $15,000 \times g$ for 10 minutes at 4 °C. Following centrifugation, the supernatant was carefully discarded, and the cell pellet was mixed with 1.5 mL of a mixture containing 25% ethanol and 96% 1 N NaOH. The resulting mixture was vigorously vortexed to ensure proper resuspension of the cell pellet. To break down the cell wall, the sample was sonicated. Subsequently, the sample under-

went another round of centrifugation at 4 °C for 15 minutes at 15,000 ×g. The supernatant obtained after centrifugation was utilized to quantify the concentration of free carbohydrates.

For the determination of lipid concentration, the sulfo-phospho-vanillin method, as described by Mishra *et al.* [22] was employed. In this method, a commercial canola oil was used as the standard. The biomass collected from the culture was subjected to the same centrifugation process as mentioned earlier. The resulting cell pellet was mixed with the appropriate reagents according to the sulfo-phospho-vanillin method, and the lipid concentration was determined.

To measure the protein content in the biomass, the bovine serum albumin standard method, as previously described by Bradford *et al.* [23], was utilized. The biomass was processed using the same centrifugation procedure mentioned above. The resulting cell pellet was subjected to protein extraction and quantification using bovine serum albumin as the standard.

2.5 Statistical Analysis

The data presented in this study are expressed as mean ± standard deviation (n = 3) for three replicates. Statistical analysis was performed using SPSS software (ver. 22.0, SPSS Inc., Chicago, IL, USA), employing one-way analysis of variance (ANOVA) followed by Duncan's multiple range post hoc test. A *p*-value less than 0.05 (*p* < 0.05) was considered statistically significant and denoted by different letters. All figures were generated using GraphPad Prism 8 software (GraphPad Software Inc., San Diego, CA, USA).

3. Results

3.1 Effect of Fungicide TM on Cyanobacteria Growth

The exposure to TM had a significant (*p* < 0.05) and dose-dependent inhibitory effect on the growth of *Spirulina* (*A. platensis*), a filamentous cyanobacterium. As the concentration of TM increased, the growth inhibition became more pronounced, indicating a strong sensitivity of *A. platensis* to this fungicide (Fig. 1A). The negative impact of TM on growth was observed throughout the entire cultivation period, with the inhibitory effect intensifying over time (Fig. 1A). After 96 hours of exposure, the growth of *A. platensis* was notably reduced by 31%, 38%, 44%, 45%, and 52% in response to TM10, TM15, TM20, TM25, and TM30 treatments, respectively, compared to the untreated control (Fig. 1A).

The IR of *A. platensis*, which reflects the extent of growth suppression caused by TM, followed a similar trend as the biomass measurements. The IR increased gradually with higher TM concentrations, indicating a dose-dependent response (Fig. 1B). After 4 days of exposure, the IR of *A. platensis* was enhanced by 29%, 35%, 40%, 41%, and 48% in the presence of TM10, TM15, TM20, TM25, and TM30, respectively (Fig. 1B).

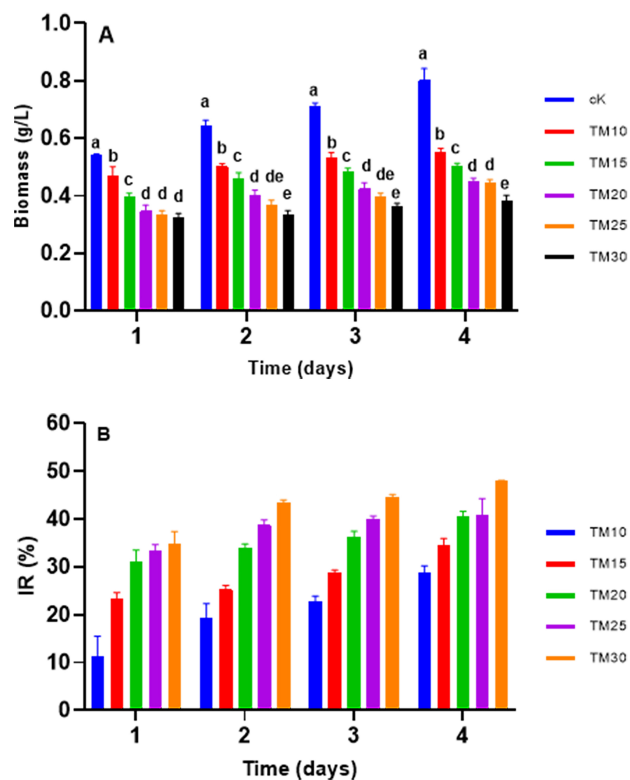


Fig. 1. Effect of thiophanate-methyl (TM) on *Arthrospira platensis* growth in terms of biomass (A) and specific growth inhibition rate (IR) (B) within 4 days of cultivation. Error bars indicate standard deviation (n = 3). Significant differences obtained statistically were indicated by different letters and styles (*p* < 0.05). The various letters a, b, c, d, e for each column bar indicate that the corresponding groups differ significantly from each other at the 5% significance level.

These findings highlight the sensitivity of *A. platensis* to TM exposure, suggesting that this fungicide can significantly impair the growth and development of this cyanobacterial species. The dose-dependent relationship observed between TM concentration and growth inhibition underscores the importance of considering the potential ecological risks associated with the use of this pesticide in aquatic environments. Further research is needed to elucidate the underlying mechanisms and specific biochemical pathways involved in the inhibitory effects of TM on *A. platensis*. Such knowledge is crucial for developing effective strategies to mitigate the detrimental impacts of pesticide residues and safeguard the health and balance of aquatic ecosystems.

3.2 Effects of Fungicide TM on Pigment Contents

The observed changes in pigment contents of *A. platensis* under the influence of TM provide valuable insights into the physiological response of this cyanobacterium to fungicide exposure. The significant decrease in chlorophyll a and b contents suggests an impairment in the

photosynthetic system of *A. platensis* caused by TM exposure (Fig. 2). Chlorophylls are crucial pigments involved in capturing light energy for photosynthesis, and their reduction indicates a disturbance in the photosynthetic process. The increase in carotenoid contents (Fig. 2), on the other hand, can be seen as a protective response of *A. platensis* to TM-induced stress. Carotenoids play a vital role in photoprotection by dissipating excess light energy and scavenging reactive oxygen species. The elevated carotenoid levels observed in this study suggest that *A. platensis* activates its defense mechanisms to mitigate the oxidative stress caused by TM exposure.

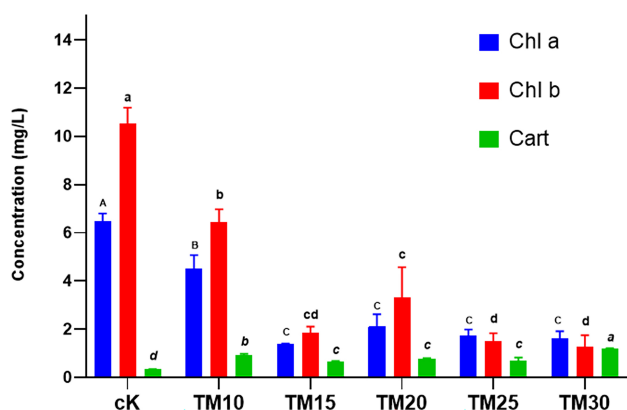


Fig. 2. Effect of TM on the contents of chlorophyll a, b and carotenoids in *Arthrospira platensis*. Error bars indicate standard deviation (n = 3). Significant differences obtained statistically were indicated by different letters and styles ($p < 0.05$). The various letters a, b, c, d for each column bar indicate that the corresponding groups differ significantly from each other at the 5% significance level.

The contrasting responses of chlorophylls and carotenoids to TM exposure highlight the complexity of the physiological adjustments occurring in *A. platensis*. These findings indicate that TM not only disrupts the photosynthetic machinery but also triggers adaptive responses aimed at maintaining cellular homeostasis and survival. Understanding the changes in pigment composition provides important insights into the overall health and functioning of *A. platensis* in response to environmental stressors like TM. These pigment-based responses can serve as indicators of the ecological health of aquatic ecosystems, as cyanobacteria are primary producers that play a crucial role in nutrient cycling and food webs.

3.3 Effect of TM on Oxidative Stress Biomarkers

In Fig. 3, the results illustrate the significant impact of TM fungicide on the induction of MDA and H_2O_2 contents in *A. platensis* at different concentrations (10–30 $\mu\text{g/L}$). The MDA content showed a significant increase across all TM concentrations compared to the control (Fig. 3A). Sim-

ilarly, the H_2O_2 content exhibited a significant increase at all tested dosages of TM, except for 10 $\mu\text{g/L}$ (Fig. 3B). The observed increase in MDA content indicates enhanced lipid peroxidation in *A. platensis* in response to TM exposure. This suggests that TM triggers oxidative stress, leading to damage in lipids and disruption of cell membranes in the cyanobacteria. The elevated H_2O_2 content suggests an imbalance between reactive oxygen species (ROS) generation and the antioxidant defense system in *A. platensis*. Specifically, following TM treatments of TM15, TM20, TM25, and TM30, the H_2O_2 content increased by 37%, 54%, 50%, and 46%, respectively, compared to the untreated control (Fig. 3B). These findings highlight that higher concentrations of TM result in a greater accumulation of H_2O_2 , intensifying oxidative stress in *A. platensis*. The significant induction of MDA and H_2O_2 contents in *A. platensis* in response to TM exposure indicates the detrimental effects on cellular health and oxidative status.

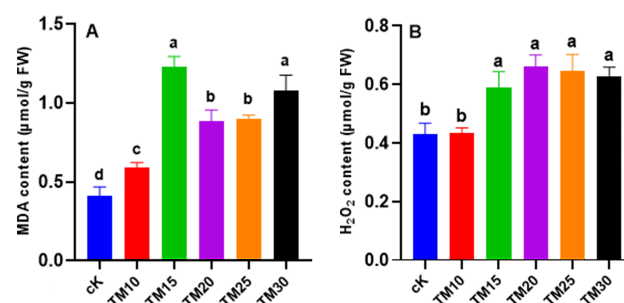


Fig. 3. Effect of TM on the contents of MDA (Malondialdehyde) (A) and H_2O_2 (B) in *Arthrospira platensis*. Error bars indicate standard deviation (n = 3). Significant differences obtained statistically were indicated by different letters ($p < 0.05$). The various letters a, b, c for each column bar indicate that the corresponding groups differ significantly from each other at the 5% significance level.

3.4 Effects of TM on Antioxidant System

In Fig. 4, the activity of APX in *A. platensis* demonstrated a significant difference ($p < 0.05$) between the control group and treatment groups. The APX activity showed a notable increase in response to TM exposure. Specifically, following TM treatments of TM10, TM15, TM20, TM25, and TM30, the APX activity in *A. platensis* increased significantly by 53%, 50%, 96%, 157%, and 43%, respectively, compared to the control group (Fig. 4A). These findings indicate the activation of the antioxidant defense system in *A. platensis* to counteract the oxidative stress induced by TM. In contrast, the activity of CAT in *A. platensis* was not significantly affected by TM10, TM15, and TM20 treatments when compared to the control group (Fig. 4B). However, significant differences ($p < 0.05$) were observed at higher concentrations of TM (25 and 30 $\mu\text{g/L}$)

compared to the control group. At these concentrations, the CAT activity in *A. platensis* was significantly increased by TM (Fig. 4B). These results suggest that CAT plays a role in the response to higher levels of TM-induced oxidative stress. The observed increase in APX activity indicates the activation of the ascorbate-glutathione cycle, which is involved in the detoxification of ROS. The significant enhancement in APX activity following TM treatments reflects the cyanobacterium's attempt to mitigate oxidative damage by increasing the activity of this antioxidant enzyme. Furthermore, the significant increase in CAT activity at higher TM concentrations suggests the involvement of CAT in the breakdown of H₂O₂ to prevent its accumulation and minimize oxidative damage. The differential responses of APX and CAT activities to TM exposure indicate the complexity of the antioxidant defense mechanisms in *A. platensis* and their modulation in response to varying TM concentrations. These findings emphasize the importance of antioxidant enzymes in protecting cyanobacteria against oxidative stress induced by pesticide residues.

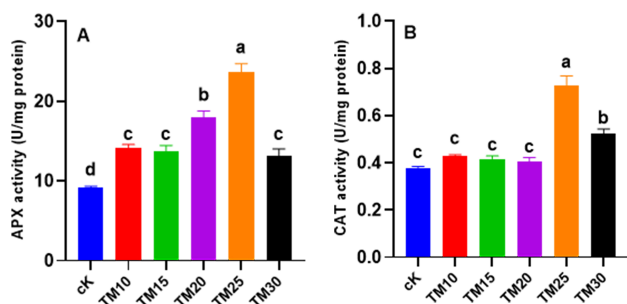


Fig. 4. Effect of TM on the activity of APX (ascorbate peroxidase) (A) and CAT (catalase) (B) in *Arthrospira platensis*. Error bars indicate standard deviation (n = 3). Significant differences obtained statistically were indicated by different letters ($p < 0.05$). The various letters a, b, c for each column bar indicate that the corresponding groups differ significantly from each other at the 5% significance level.

3.5 Effects of TM on Detoxifying Enzymes

In Fig. 5, the activity of GST in *A. platensis* was significantly ($p < 0.05$) affected by exposure to the fungicide TM. Notably, higher dosages of TM (20, 25, and 30 $\mu\text{g/L}$) resulted in a further stimulation of GST activity, indicating a dose-dependent response to fungicide stress (Fig. 5A). These findings highlight the role of GST in the detoxification and metabolism of TM in *A. platensis*. Similarly, the activity of POD exhibited a similar trend in response to TM exposure. *A. platensis* exposed to concentrations of 10, 15, 20, 25, and 30 $\mu\text{g/L}$ of TM showed a significant increase in POD activity compared to the control group (Fig. 5B). This increase in POD activity suggests the involvement of this enzyme in the detoxification of ROS generated as a result

of TM exposure. The observed dose-dependent responses of GST and POD activities provide insights into the adaptive mechanisms employed by *A. platensis* to cope with fungicide-induced stress. The enhanced activities of GST and POD indicate the activation of defense mechanisms to counteract the potential toxic effects of TM and maintain cellular homeostasis. Overall, these findings highlight the importance of GST and POD activities in the detoxification and stress response of *A. platensis* to fungicide TM exposure, emphasizing the need for further research on the mechanisms underlying these responses and their ecological implications in aquatic ecosystems.

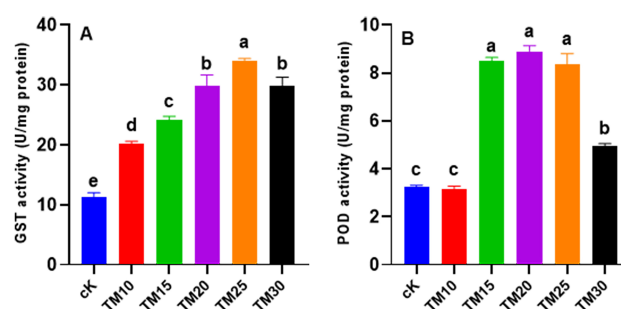


Fig. 5. Effect of TM on the activity of GST (glutathione-S-transferase) (A) and POD (peroxidase) (B) in *Arthrospira platensis*. Error bars indicate standard deviation (n = 3). Significant differences obtained statistically were indicated by different letters ($p < 0.05$). The various letters a, b, c, d, e for each column bar indicate that the corresponding groups differ significantly from each other at the 5% significance level.

3.6 Effects of TM on Primary Biocomponents

In Fig. 6, the effects of TM fungicide on the levels of various bio-components in *A. platensis* are presented. Exposure to TM at concentrations ranging from 10 to 30 $\mu\text{g/L}$ resulted in significant ($p < 0.05$) reductions in the content of several macromolecules, including carbohydrates and proteins, compared to the control group (Fig. 6). Specifically, the carbohydrate content in *A. platensis* decreased with increasing concentrations of TM (Fig. 6A). Significant decreases of 15%, 10%, 45%, 60%, and 38% in protein content were observed at TM concentrations of 10, 15, 20, 25, and 30 $\mu\text{g/L}$, respectively, relative to the control (Fig. 6B). In contrast, the lipid content exhibited a significant ($p < 0.05$) increase in response to TM exposure, except at the relatively lower concentration of 10 $\mu\text{g/L}$. The highest lipid content (112%) was recorded at TM concentration of 25 $\mu\text{g/L}$ compared to the control (Fig. 6C). These findings suggest that TM fungicide has a detrimental impact on the bio-component composition of *A. platensis*. The decrease in carbohydrate and protein contents indicates an impairment of cellular metabolic processes, likely resulting from the toxic effects of TM. On the other hand, the increased lipid

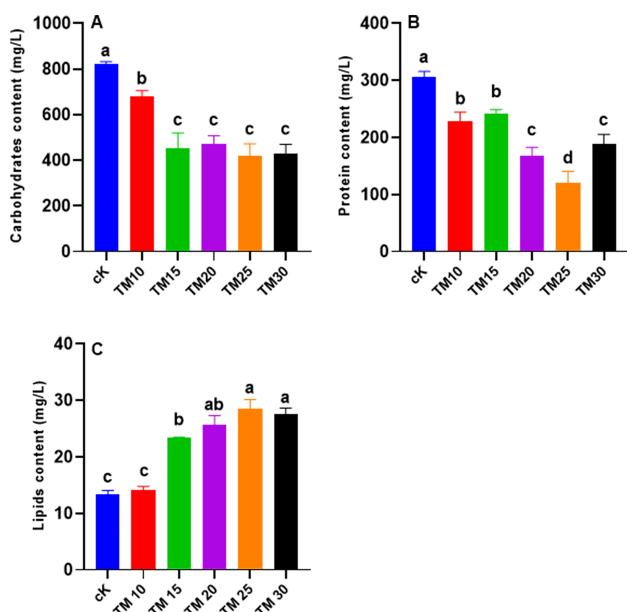


Fig. 6. Effect of TM on the contents of carbohydrates (A), proteins (B) and lipids (C) in *Arthrospira platensis*. Error bars indicate standard deviation ($n = 3$). Significant differences obtained statistically were indicated by different letters ($p < 0.05$). The various letters a, b, c, d, ab for each column bar indicate that the corresponding groups differ significantly from each other at the 5% significance level.

content may be a response to TM-induced stress, as lipids can serve as energy reserves and play a protective role in cellular membranes.

4. Discussion

Several studies have investigated the effects of pesticides on freshwater microalgae, highlighting the potential ecological risks associated with these chemical contaminants [24–26]. However, limited research has been conducted on the specific impact of fungicides on cyanobacteria, such as *Arthrospira platensis*. In this study, we aimed to fill this knowledge gap by investigating the effects of TM, a common fungicide found in aquatic ecosystems, on the growth, biocomponents, and oxidative stress responses of *A. platensis*. Our findings revealed a clear and significant impact of TM exposure on the biomass of *A. platensis*, indicating a direct inhibition of growth (Fig. 1A). Interestingly, this growth inhibition exhibited a dose-dependent relationship with TM concentrations and was also influenced by the duration of exposure (Fig. 1B). These results are consistent with previous studies that have reported similar inhibitory effects of pesticides on microalgae [27]. For instance, studies have demonstrated the high toxicity of glyphosate to cyanobacteria such as *Pseudanabaena sp.* [28]. Furthermore, recent research has highlighted the negative effects of microplastics, particularly polyethylene, on the growth of *spirulina* [11]. The observed growth inhibition can be

attributed to the excessive production of ROS as a result of membrane lipid damage caused by xenobiotic compounds [29]. This oxidative stress disrupts essential processes such as chlorophyll synthesis, ultimately leading to decreased biomass production. Additionally, we observed that the IR increased with prolonged exposure and higher concentrations of TM fungicide. Notably, the highest IR was observed after 96 hours of TM30 treatment, indicating a more pronounced growth inhibition effect at higher concentrations (Fig. 1B). These findings are consistent with a study conducted by Dong *et al.* [13], which demonstrated similar concentration-dependent effects of sulfamethoxazole (SMZ) on *Chlorella vulgaris* and *Microcystis aeruginosa*.

Chlorophyll pigments are crucial for the process of photosynthesis in microalgae [30]. Chlorophyll a, in particular, plays a key role in capturing light energy and converting it into chemical energy [31]. The significant decrease in chlorophyll a and b contents observed in *A. platensis* exposed to TM fungicide indicates a disturbance in the photosynthetic capacity of the microalgae (Fig. 2). This reduction in chlorophyll levels can be attributed to the negative effects of TM on the photosynthetic apparatus, such as damage to the electron transfer system of PSII and the accumulation of ROS [32]. These findings align with previous studies that have reported decreases in chlorophyll content in microalgae exposed to different pollutants [10,14,33]. Furthermore, the positive correlation between chlorophyll a content and biomass highlights the close relationship between photosynthetic activity and the growth of *A. platensis* (Figs. 1 and 2). The decline in chlorophyll pigments due to TM exposure can have a detrimental impact on the overall productivity and biomass accumulation of the cyanobacteria. This suggests that TM fungicide poses a potential hazard to the growth and development of *A. platensis*. On the other hand, carotenoids play a protective role in microalgae by absorbing excessive light energy and quenching ROS, thereby preventing oxidative damage to the photosynthetic apparatus [31]. In our study, the increased content of carotenoids in response to TM exposure indicates a compensatory mechanism employed by *A. platensis* to counteract the harmful effects of the fungicide (Fig. 2). Carotenoids act as antioxidants and help maintain the stability of photosynthetic performance, protecting the microalgae from oxidative stress caused by TM exposure. These findings are consistent with previous research that has demonstrated the ability of certain contaminants to enhance carotenoid levels in microalgae, thus improving their antioxidant capacities [14]. Overall, the significant decrease in chlorophyll content and the concurrent increase in carotenoid content highlight the adverse effects of TM fungicide on the photosynthetic activity of *A. platensis*. These findings underscore the importance of assessing the impacts of environmental contaminants on microalgae, as they serve as vital components of aquatic ecosystems and play a significant role in global biogeochemical

cycles [34]. Understanding the responses of microalgae to contaminants is crucial for evaluating the ecological implications and developing strategies for environmental management and conservation.

Exposure to aquatic xenobiotics can disrupt the balance of ROS in freshwater microalgae, resulting in oxidative stress [32]. A byproduct of lipid peroxidation is commonly used as a biomarker to evaluate the degree of oxidative stress induced by pollutants such as fungicides [35]. In our study, we observed a significant increase in MDA content in *A. platensis* (Fig. 3A), indicating that TM stress adversely affected the integrity of cell membranes and led to oxidative damage to unsaturated fatty acids [14]. The elevated MDA levels observed can be attributed to the excessive generation of ROS following exposure to TM fungicide (Fig. 3B). These findings align with a previous study that reported increased MDA contents in the cyanobacterium *Nostoc muscorum* under captan fungicide stress [35]. The measurement of MDA serves as a valuable tool for assessing oxidative stress and lipid peroxidation in microalgae exposed to xenobiotics. Increased MDA levels indicate the occurrence of oxidative damage and disruption of membrane integrity, which can have significant implications for the overall health and physiological functions of microalgae. By quantifying MDA, we gain insights into the extent of oxidative stress induced by TM fungicide in *A. platensis*, shedding light on the potential risks posed by this pollutant to aquatic ecosystems. It is important to recognize that the generation of ROS and subsequent oxidative stress are pivotal mechanisms underlying the toxic effects of xenobiotics on microalgae. Oxidative stress can lead to cellular damage, impaired growth, and reduced photosynthetic efficiency, ultimately impacting the overall health and survival of microalgae populations. Monitoring and understanding the oxidative stress response in microalgae are crucial for assessing the ecological impacts of pollutants and developing strategies to mitigate their adverse effects on aquatic environments.

Cyanobacteria, including *Spirulina*, have evolved a sophisticated enzymatic antioxidant defense system to counteract the oxidative damage induced by pollutants [13, 32]. One of the key antioxidant enzymes in this system is catalase (CAT), which plays a vital role in converting H_2O_2 into harmless water (H_2O) and oxygen (O_2) molecules [13]. In our study, we observed a significant increase in CAT and APX activity in *Spirulina* exposed to TM fungicide (Fig. 4). The enhanced CAT activity can be attributed to the augmented production of H_2O_2 , a principal ROS involved in various fundamental biological processes. This upregulation of CAT activity may be a result of the activation of existing enzyme pools or the upregulation of CAT-encoding genes, triggered by the elevated levels of H_2O_2 [36]. Numerous studies have demonstrated similar increases in CAT and APX activities following exposure to different contaminants [25]. For example, the activity of CAT was signifi-

cantly elevated in *Chlorella pyrenoidosa* and *Merismopedia sp.* exposed to Chlorpyrifos [37]. The induction of CAT and APX activities in response to TM exposure underscores the cyanobacteria's adaptive antioxidant defense mechanisms, which enable them to counterbalance oxidative stress. These enzymes play crucial roles in detoxifying and scavenging ROS, thereby safeguarding the cells against oxidative damage. The observed upregulation of CAT activity in our study aligns with previous research findings, highlighting the robustness of this antioxidant defense system across various microalgae species when exposed to pollutants.

GST is a crucial enzyme involved in the detoxification of xenobiotics by catalyzing the conjugation reaction with glutathione (GSH) in phase II of xenobiotic metabolism [33]. In our study, we observed that the catalytic activity of GST in *A. platensis* was significantly stimulated with increasing TM dosage (Fig. 5A). This dosage-dependent trend in the detoxification process coincided with the IR observed for the respective TM dosages (Fig. 1B). These findings indicate that higher TM dosages had a greater impact and induced a stronger detoxification response in *A. platensis*. Moreover, our results provide evidence that fungicide pollution poses significant challenges to cyanobacteria, which respond by metabolizing the contaminants through phase II conjugation with GSH [35]. These findings are consistent with the study conducted by Hamed *et al.* [38], where greater catalytic activities of GST were observed in *Scenedesmus obliquus* and *Nostoc muscorum* under propiconazole treatment, supporting our present data (Fig. 5A). In addition to GST, we observed an induction of POD activity in *A. platensis* following exposure to TM, showing dose-dependent responses under fungicide stress (Fig. 5B). The enhanced POD activity due to TM exposure might play a vital role in the transformation of xenobiotics [38]. Several studies have investigated the impact of pesticides on cyanobacterial detoxification enzymes, revealing their involvement in the pathways of xenobiotic detoxification and elimination. For instance, Yisa *et al.* [39] found that the catalytic activity of GST and POD in *Microcystis* was promoted by the amoxicillin antibiotic, indicating their participation in xenobiotic detoxification processes.

Biochemical macromolecules, including carbohydrates, lipids, and proteins, play crucial roles in the adaptation of organisms to environmental stressors, such as pollutants [14,40]. In our study, we observed significant changes in the contents of carbohydrates, proteins, and lipids in *A. platensis* following exposure to the fungicide TM (Fig. 6A,B and C). These alterations in macromolecule levels provide insights into the adaptive responses of cyanobacteria to xenobiotic stress. The decrease in protein content observed in *A. platensis* can be attributed to the catabolism of proteins into amino acids, which serve as an energy source to combat the adverse effects of fungicide stress [40]. Similarly, the reduction in carbohydrate content

suggests a disruption in carbohydrate synthesis pathways, potentially affecting the energy metabolism of cyanobacteria (Fig. 6A). This finding aligns with previous studies that reported decreases in protein and carbohydrate contents in *spirulina* exposed to antibiotic treatments [10]. In contrast to proteins and carbohydrates, the lipid content in *A. platensis* showed an increase following TM exposure (Fig. 6C). This elevation in lipid accumulation can be linked to the enhanced generation of ROS triggered by TM exposure (Fig. 3B). Cyanobacteria may utilize lipids as a defense mechanism, as lipids can absorb organic xenobiotics and restrict their bioavailability, thereby mitigating their toxic effects [10,40]. This phenomenon has also been observed in other studies, such as the significant lipid accumulation in *Chlorella sp.* exposed to the insecticide alpha-cypermethrin [41]. Moreover, the changes in lipid content reflect the modulation of lipid metabolism in response to membrane damage caused by fungicide exposure (Fig. 3A). The promotion of lipid metabolism supports membrane repair processes and helps maintain the integrity and functionality of the cell [10]. The disturbances in carbohydrate, protein, and lipid contents in *A. platensis* indicate the disruption of energy reserve synthesis and metabolic processes caused by fungicide exposure [42]. These alterations are indicative of the cyanobacteria's attempts to cope with xenobiotic-induced stress. However, further investigations are warranted to elucidate the underlying mechanisms involved in the adaptive responses of cyanobacteria to environmental pollutants.

Overall, this study provides valuable insights into the effects of the fungicide TM on the growth, biocomponents, and oxidative stress responses of the cyanobacterium *Arthrospira platensis*. The results demonstrate a clear and significant inhibition of growth, which is dose-dependent and influenced by the duration of exposure. The decrease in chlorophyll content and the concurrent increase in carotenoid content indicate disturbances in the photosynthetic capacity of *A. platensis* and highlight the potential hazards posed by TM fungicide. Additionally, the elevation of MDA content suggests oxidative damage and disruption of membrane integrity, emphasizing the role of oxidative stress in the toxic effects of TM. The upregulation of antioxidant enzyme activities, such as catalase, ascorbate peroxidase, and glutathione-S-transferase, indicates the activation of adaptive defense mechanisms against oxidative stress and xenobiotic detoxification. Furthermore, the changes in carbohydrate, protein, and lipid contents reflect the adaptive responses of *A. platensis* to fungicide-induced stress, involving energy reserve utilization and modulation of lipid metabolism. These findings contribute to the understanding of the ecological risks associated with fungicide pollution and the adaptive mechanisms of cyanobacteria in response to environmental contaminants. It is crucial to assess the impacts of such pollutants on microalgae, considering their significance in aquatic ecosystems and global biogeochem-

ical cycles. The evaluation of oxidative stress responses and the activity of detoxification enzymes in microalgae are essential for the assessment and management of environmental pollutants. Further research is needed to unravel the underlying mechanisms and pathways involved in the adaptive responses of cyanobacteria to fungicides and other xenobiotic compounds, facilitating the development of effective strategies for environmental conservation and management.

5. Conclusions

In the present study, TM fungicide was found to have significant and detrimental effects on *Arthrospira platensis*, a cyanobacterium commonly known as *spirulina*. One of the key findings of our study is the generation of ROS and lipid peroxidation in *A. platensis* following TM exposure. The observed lipid peroxidation indicates a disruption of the cell membrane's integrity, which can have severe consequences for the overall health and functionality of the cyanobacteria. Furthermore, TM exposure led to alterations in the antioxidant and detoxification enzymatic responses of *A. platensis*. Another significant impact of TM on *A. platensis* is the reduction in pigment content, particularly chlorophyll. Additionally, our study revealed changes in the macromolecule contents of *A. platensis* following TM exposure. Specifically, carbohydrates and proteins showed a significant decrease, while lipid accumulation increased. These alterations in macromolecule levels reflect the adaptive adjustments made by *Spirulina* in response to TM-induced stress. To gain a deeper understanding of the molecular mechanisms underlying the observed effects, future studies employing transcriptomic and metabolomic approaches are warranted. This article contributes significantly by delving into the intricate relationship between pesticide residues, particularly TM, and *Arthrospira platensis* in aquatic ecosystems. It provides valuable insights into the potential ecological hazards posed by these residues and emphasizes the urgency of adopting sustainable agricultural practices to safeguard the health of these precious aquatic environments.

Availability of Data and Materials

The data and material for the current study are included in this published article. There is no other data generated nor used. The datasets used and/or analyzed during the present experiments are available from the corresponding author on reasonable request.

Author Contributions

NT, MA, CB, RA, AD, ZB, HT, JZ, AA: Performed experimental work. AA, NT, AHHB and HT: Supervision, Conceptualization, Writing — original draft. AA, JZ and MA: Co-Supervision. NT, AHHB, HT, JZ, and AA: Writing — review & editing. NT: Data Analysis. NT reviews

excel and graphs. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work. All authors contributed to editorial changes in the manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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